EUR. J. ONCOL.; Vol. 19, n. 3, pp. 159-165, 2014

Intraoperative radiotherapy during breast cancer surgery: acute and chronic cardiac safety tested by an ultra-sensitive troponin and N-terminal Pro-B-type natriuretic peptide

Tania Saibene[1], Silvia Michieletto[1], Laura Evangelista[2], Alessandra Bianchi[3], Enrico Orvieto[4], Ornella Lora[2], Franco Berti[2], Fernando Bozza[1], Alberto Banzato[3]

[1] Oncological Surgery Unit, Veneto Institute of Oncology IOV - IRCCS, Padua, Italy; [2]Radiotherapy and Nuclear Medicine Unit, Veneto Institute of Oncology IOV - IRCCS, Padua, Italy; [3] Cardiology Unit, Veneto Institute of Oncology IOV - IRCCS, Padua, Italy; [4] Department of Pathology, University Hospital of Padua, Padua, Italy

Summary. *Aim:* Intraoperative radiotherapy (IORT) is now an acceptable option for low risk early stage breast cancers, allowing patients not to undergo a full course of external radiotherapy. It is not clear, however, whether IORT, providing radiations very close to the chest wall, may produce heart damage. In this study we tried to evaluate if acute or chronic heart damage in early stage breast cancer patients treated with breast conservative surgery (BCS) and IORT, can be assessed by ultra-sensitive cardiac Troponine I (TnI) levels or N-terminal proB-type natriuretic peptide (NT-proBNP), respectively. *Materials and methods:* We enrolled 43 patients who received IORT for breast cancer, as part of a TARGIT-A trial. Twenty-two patients had left and 21 right breast cancer. TnI levels were measured immediately before surgery, and six hours after the end of IORT; both TnI and NT-proBNP levels were measured after 12 months. For the patients with left breast cancer, a little tungsten sheet was placed under the major pectoralis muscle, on the chest projection of the X-ray source, in order to protect the heart. *Results:* None of the patients showed altered serum levels of TnI before surgery, or after IORT procedure and NT-proBNP during follow-up. No difference in TnI and NT-proBNP values was noticed between right-sided and left-sided breast cancers treated with BCS and IORT. *Conclusions:* IORT for early stage breast cancer treatment does not increase TnI and NT-proBNP levels, suggesting that the procedure does not determine acute or chronic heart damage.

Key words: intraoperative radiotherapy; breast cancer; troponin I; N-terminal ProB-type natriuretic peptide; breast conservative surgery

«Radioterapia intraoperatoria durante la chirurgia del cancro della mammella: effetti acuti e cronici a livello cardiaco testati mediante troponina ultra-sensibile e peptide Pro-B natriuretico N-terminale»

Riassunto. *Scopo:* La radioterapia intraoperatoria (IORT) è considerata una opzione accettabile per i tumori della mammella allo stadio iniziale a basso rischio, in quanto evita ai pazienti di dover sopportare un ciclo completo di radioterapia esterna. E' tuttavia non chiaro se la IORT, erogando radiazioni molto vicine alla parete del torace, produca danni al cuore. In questo studio si vuole valutare se danni acuti o cronici a livello cardiaco in pazienti con tumore mammario allo stadio iniziale trattati con chirurgia mammaria conservativa (BCS) e IORT, possano essere valutati attraverso livelli supersensibili di Troponina I cardiaca e peptide Pro-B natriuretico N-terminale (NT-proBNP). *Materiali e metodi:* Abbiamo arruolato 43 pazienti che hanno ricevuto la IORT per cancro al seno, facenti parte dello studio clinico TARGIT-A. Ventidue pazienti avevano

il cancro al seno sinistro e 21 al seno destro. I livelli di TnI sono stati misurati immediatamente prima della chirurgia e 6 ore dopo la fine della IORT; inoltre, sia i livelli di TnI e NT-proBNP sono stati misurati dopo 12 mesi. Per i pazienti con cancro al seno sinistro, è stato posizionato sotto il muscolo pettorale maggiore, un foglio di tungsteno, sulla proiezione pettorale della sorgente di raggi X, al fine di proteggere il cuore. *Risultati:* Nessuno dei pazienti ha mostrato livelli di siero alterati del TnI prima della chirurgia, e dopo la procedura di IORT e NT proBNP durante il follow-up. Non è stata notata nessuna differenza nei valori TnI e NT-proBNP tra il cancro al seno destro ed al seno sinistro trattati con BCS e IORT. *Conclusioni:* La IORT per il trattamento del cancro alla mammella in stadio iniziale non aumenta i livelli di TnI e NT-proBNP, e suggerisce che tale procedura non determina danni al cuore di tipo acuto o cronico.

Parole chiave: radioterapia intraoperatoria, cancro della mammella, troponina I, peptide Pro-B natriuretico N-terminale, chirurgia conservativa della mammella

Introduction

Breast cancer is the most common cancer and the leading cause of cancer death among women worldwide. Breast conservative surgery (BCS) followed by adjuvant radiotherapy (RT) yields satisfactory aesthetic results without compromising local control and is now considered as the standard of care for most patients with early stage breast cancer. Nowadays, the most commonly used schedule for external beam radiation therapy (EBRT) after BCS is 45 to 50 Gray (Gy) delivered over 5-6 weeks in 1.8-2Gy daily fractions, followed by an additional boost to the tumor bed of 10-16Gy over 1-2 weeks. Even though lower than initially reported, nonetheless radiation therapy is associated with an increased risk of cardiac dysfunction (1).

In the last few years, the idea that a single dose of radiation delivered during the surgical operation, on the tumor site, could be as efficient as EBRT has been tested as a way to provide less discomfort and better cosmetic results (1). A first study performed by Vaidya *et al.* (2) showed that an intraoperative radiotherapy boost was feasible with good cosmetic results and low rates of local relapses in selected low-risk patients, thus providing the rationale for a randomized trial versus external radiotherapy. The randomized study, called TARGIT-A (3), evaluated a single dose of 20Gy delivered intraoperatively by an intrabeam device (called intraoperative radiotherapy-IORT) versus whole breast external beam radiotherapy and showed comparable local control rates in a selected patient population. This study has been recently updated, confirming the comparable efficacy of the two techniques in local control rates (4). Of interest, the TARGIT-A trial has also shown that the number of cardiac deaths, although low overall, were even less in the target intraoperative arm (4). Apart from clinical cardiac toxicities, there are very few prospective data on sub-clinical radiotherapy-induced cardiac toxicity. In fact, markers of cardiac toxicity are available. Cardiac troponin (Tn) I and T are structural proteins unique to the heart. Detection of Tn in peripheral blood indicates cardiomyocyte necrosis and is the most widely used biomarker for detection of myocardial damage (5). Although widely used to detect myonecrosis in the setting of ischemia, Tn is also elevated with other acute and chronic disease processes, including heart failure, pulmonary embolic disease, renal failure, sepsis, chemotherapy injury and others. Natriuretic peptides, such as N-terminal pro B-type (NT-proBNP), are secreted from the heart in response to cardiac hemodynamic stress mediated by volume and/or pressure overload. Although non cardiac specific, these latter biomarkers are a sensitive tool of cardiac dysfunction. Elevated levels can be detected early in the asymptomatic stage of heart disease or in patients with preserved ejection fraction.

There are only a few studies of cardiac biomarkers in patients undergoing EBRT and these studies have shown conflicting results (6-9). The aim of this study was to investigate, by ultra-sensitive TnI plasma and NT-proBNP levels, the acute and chronic safety of IORT during early stage breast cancer surgery.

Methods and materials

Study Design

At the Veneto Institute of Oncology, patients with Early Breast Cancer who were candidates for Breast Conservative Surgery and IORT in the context of the TARGIT-A protocol (3) were enrolled into a prospective study designed to evaluate the cardiac safety of the procedure. All patients reporting a personal history of cardiac disorder (cardiomyopathy, ischemic heart disease, valvulopathies, and arrhythmias) were excluded from selection. Between July 2011 and July 2012, 43 patients enrolled in the TARGIT-A trial were selected for the cardiac safety study; 21 patients with right and 22 with left breast cancers. As reported in Table 1, the median age of the population was 61.4 yrs; most of the tumors were Invasive Ductal Carcinoma (n=38; 88.4%), with positive hormone receptor (n=26, 60.5%), negative Her-2 (n=40, 93.2%), Grade 2 (n=22, 51.2%), MIB-1 <20% (n=22, 51.2%). All patients had a preoperative cardiologic evaluation by electrocardiography (ECG), and transthoracic echocardiography. Five patients had a history of blood hypertension treated by antihypertensive drugs. The study was conducted according to the Declaration of Helsinki and was approved by the hospital's ethics committee. All patients provided written informed consent.

Surgical and radiotherapeutic treatment

The IORT treatment, according to the TARGIT-A protocol (3), delivered all required radiation in a single fraction during the surgical stage. The Intrabeam (Carl Zeiss Meditec) device provided a source point of low energy x-rays at the tip of a 3.2 mm diameter tube that was placed at the centre of a spherical tumor bed applicator. After excision of the tumor, the surgical specimen was macroscopically examined by a pathologist, to evaluate the lump size and the margins, in order to decide if the surgery performed was sufficient or immediate re-excision of the margins needed to be performed to achieve clear margins. The sentinel lymph node was examined immediately on frozen sections and an immediate axillary dissection was performed if found positive.

Table 1. Characteristics of the patient population

Characteristics	
Median age (range), yrs	61.42 (44-76)
Histological type	
IDC	38 (88.4%)
ILC	3 (7%)
Others	1 (2.3%)
Unknown	1 (2.3%)
Clinical staging	
cT1aN0	5 (11.6%)
cT1bN0	13 (30.2%)
cT1cN0	13 (30.2%)
cT1bN1	3 (7%)
cT1cN1	4 (9.4%)
cT1cN2	1 (2.3%)
cT2cN1	1 (2.3%)
unknown	3 (7%)
Hormone receptors	
ER+PR+	26 (60.5%)
ER+PR-	12 (28%)
ER-PR-	3 (7%)
unknown	2 (4.5%)
Her 2-neu	
Negative	40 (93.2%)
Positive	2 (4.5%)
Unknown	1 (2.3%)
Side of disease	
Left	22 (51.2%)
Right	21 (48.8%)
Grading	
G1	13 (30.2%)
G2	22 (51.2%)
G3	5 (11.6%)
unknown	3 (7%)
MIB-1	
<20%	22 (51.2%)
>20%	10 (23.2%)
unknown	11 (25.6%)

IDC: invasive ductal cancer; ILC: invasive lobular cancer; ER: estrogen receptor, evaluated by immunohistochemistry method; PR: progesteron receptor, evaluated by immunohistochemistry method.

An Intrabeam spherical applicator was adapted to the size of the cavity left by the tumor excision. The applicator was placed on the tumor bed, and the breast parenchyma was stretched close to the spherical appli-

cator and closed over it with stitches. Then, radiation was switched on for 25-30 minutes to target the area at high risk of local recurrence. The surface of the tumor bed typically received 20Gy, which falls to 5-7Gy at 1 cm depth. In order to protect the heart from radiation damage, when IORT was performed on the left breast, a little sheet of tungsten, 4-5 cm diameter, was placed under the major pectoralis muscle on the chest projection of the spherical applicator by dissecting the muscle tissue which was then sutured over the tungsten sheet in order to achieve a good irradiation of the muscle too. At the end of the procedure, the tungsten sheet was removed and the muscle repaired with stitches.

TnI and NT-proBNP essays

In patients who were selected for IORT, "basal" serum TnI levels were obtained on the same day as the operation and 6 hours after breast conservative treatment plus IORT. Then, 12-months after surgery, TnI and NT-proBNP measurements were repeated, and a new ECG was performed. Venous blood samples were collected into Vacutainer® tubes containing lithium heparin. Plasma samples were obtained shortly after venipuncture by centrifugation and assayed immediately. Analyses of TnI and of NT-proBNP were determined using a commercially available LOCI® (Luminescent Oxygen Channelling Immunoassay) by Dimension® EXL Analyzer (Siemens Healthcare Diagnostics, Newark, NY, USA). The analytical range for TnI was 0.015-40.000 μg/L; 10% CV <0.04 μg/L; 99° percentile (critical level for heart damage) =0.045 μg/L. The analytical range for NT-proBNP was 5.0-35.000.0 ng/L; CV=2.2-9.01%.

Statistical analysis

Continuous data are presented as medians (range) and categorical data as percentages.

Results

In all 43 patients the basal serum level of TnI was <0.017 μg/L. None of the 43 patients showed an increase in serum TnI levels after local excision of the tumor and IORT, thus registering levels of TnI lower than 0.017 μg/L. Among the patients treated for left breast cancer, independently of the quadrant (outer or inner), no increase in TnI levels was detected. Moreover, neither different times of exposure to IORT nor spherical applicators of different sizes influenced TnI levels. Twelve months after surgery, all 43 patients had negative TnI values (<0.017 μg/L), and 42 patients showed negative NT-proBNP levels (median value: 69; range: 25-242 ng/L). One patient, a 70-year woman already treated by five antihypertensive drugs, reported an increase in NT-proBNP (value=526 ng/L) although within the range of normality (0-900 ng/L); at a subsequent evaluation, this patient had an NT-proBNP value of 389 ng/L and echocardiography showed normal systolic and diastolic function (ejection fraction: 63%).

Discussion

Physiopathology of radiation-induced heart damage

Radiation-induced heart disease was initially studied in patients treated with high-dose radiation schemes for Hodgkin's disease, showing an increase in the amount of fibrous tissue in the pericardium, myocardium and endocardium, and severe atherosclerosis of the coronary arteries, always confined to the irradiated volume (10). There are various radio-sensitive structures within the heart, but the myocardial damage is mainly the consequence of vascular injuries. Cardiac tolerance to radiation depends on the amount of the myocardium irradiated, and it decreases with an increasing percentage of the heart volume involved (6).

The relative risk of death from a fatal myocardial infarction in patients treated with mediastinal EBRT is 1.5 to 3.0 in comparison to not irradiated patients (11, 12). In young patients undergoing mediastinal irradiation, the prevalence of myocardial ischemia and coronary artery disease is high (13). A recent meta-analysis of eight randomized trials found a 62% increase in cardiac deaths among women who were treated with EBRT (14). Even at lower radiation doses, there appears to be an excess risk of cardiovascular disease as shown in the Japanese atomic bomb

survivors (15). The process of radiation-related heart damage starts as initial endothelial damage, which leads to acute inflammatory reaction and activation of the coagulation mechanisms with fibrin deposition, secretion of cytokines such as tumor necrosis factor, interleukin (IL)-6 and IL-1, platelet-derived growth factor, transforming growth factor-β, and subsequent fibrin formation, endothelial proliferation and collagen deposition, with enhanced atherosclerosis. Myocardial damage is characterized by non-specific, diffuse interstitial fibrosis, which alters the compliance of the myocardium and contributes primarily to diastolic dysfunction (16). Although the physiopathology of radiation-induced heart damage is well known, the precise incidence of clinical heart damage is less clear. There are many confounding factors that can explain the discordant results as to the cardiotoxicity of EBRT. First, a long follow-up is needed, to establish a reliable incidence of cardiac events attributable to RT. Second, given the frequency of coronary artery disease in Western societies, large numbers of patients are required for detection of statistically significant differences in the ratio of observed to expected events. Third, competing causes of death are likely to obscure any increase in cardiac mortality seen after EBRT (17). Finally, many of these patients receive additional therapies (chemotherapy, endocrine therapy, biological therapy) and each of these can contribute to cardiac toxicity. It is however clear that there are still concerns about the possible damage resulting from low-dose radiation exposure to the heart, as occus during EBRT or in the case of IORT during BCS.

Markers of acute cardiac damage

Potential methods of early cardiac damage assessment include biochemical tests, functional studies and scintigraphic evaluation of myocardial perfusion. Among the biochemical markers, three main molecules have been mainly considered: Tn, the atrial natriuretic peptide (ANP) and the brain natriuretic peptide (BNP).

Tn is a widely accepted marker of acute heart damage, and has also been studied as a marker of cardiotoxicity induced by cytotoxic chemotherapy (6), but it seems not to be a good marker in the follow-up of EBRT-treated patients (8, 18). Tn leaks out of injured myocardial cells through their damaged cell membranes into the bloodstream within 4 to 6 hours of a myocardial infarction and remains elevated for up to 2 weeks (19, 20). For this reason, Tn might be the best marker to evaluate cardiac acute damage after IORT.

There are only a few studies of biomarkers in patients undergoing EBRT and these studies have shown conflicting results. In a study by Hughes-Davies *et al.* (7), pre-and post-treatment serum TnT values in 50 patients with early breast cancer undergoing EBRT to the entire left breast following BCS were compared. No changes in TnT concentrations were found after 45 to 46Gy whole-breast irradiation. All women had undetectable or normal TnT levels on the first and last day of treatment. There was no evidence of any upward trend during treatment.

In another study, in 30 patients receiving thoracic chemo-radiation therapy no significant elevation in creatine kinase-myocardial band (CK-MB), Tn or NT-proBNP was detected with radiation therapy (18).

Nellessen *et al.* (6) sought to determine whether radiation treatment to the mediastinum and breast lead to the release of cardiac biomarkers. The study included 23 patients: 18 with lung cancer and 5 with breast cancer. Radiation therapy was administered for up to 6 weeks. The total radiation dose was >45Gy in each patient. Blood samples to determine TnI and BNP were taken before and once a week during radiation therapy. Both TnI and BNP increased significantly during the study; however, the absolute and mean values of both biomarkers remained on a relatively low level (mean pre-radiation and post-radiation TnI: 0.007±0.008, 0.014±0.01 ng/mL; mean pre-radiation and post-radiation BNP: 123±147, 159±184 pg/mL).

A recent study by Erven *et al.* (9) involving 75 women who received adjuvant RT to the breast/chest wall and regional lymph nodes reported significantly elevated TnI levels after RT compared with baseline in the left-sided group, whereas in the right-sided group no change in TnI levels could be noticed. However, in all patients the TnI values remained below the quantification level. Nevertheless, the authors concluded that the assay employed for TnI was not sensitive enough. In addition, as stated by D'Errico *et al.* (8), NT-proBNP plasma values were correlated with high doses of

radiation in small volumes of heart and ventricle experiencing cardiac injury.

To our knowledge, there are no data on the possible heart damage induced by partial breast irradiation performed by an Intrabeam device during surgery directly on the tumor site. In our pilot-study we evaluated the possible cardiac damage induced by IORT on 43 patients, by measuring the TnI levels as markers of acute and NT-proBNP as marker of chronic injury respectively. We used ultra-sensitive cardiac troponin in accordance with Erven *et al.* (9), to confirm the differences in TnI levels in the early detection of post-RT cardiotoxicity.

The distance between the left ventricle and chest wall determines the percentage of myocardium involved in the radiation field (8), and can be predictive of the myocyte damage induced by an X-ray tangential field like the one used during EBRT; this can be even more true when the radiotherapy source is very close to the chest and producing a spherical X-ray field. During IORT, in fact, the X-ray source is placed directly inside the tumor excision cavity, in contact with the chest wall, and X-rays spread around, irradiating the major pectoralis muscle and possibly the heart. In our patients, in order to protect the chest wall and the heart from radiation, in the 22 left-sided breast cancers a little sheet of tungsten was placed under the major pectoralis muscle. Interestingly, we found no difference in TnI serum levels between patients with left breast cancer and right breast cancer. TnI is a uniquely sensitive marker of myocyte membrane integrity, and the normal values observed during treatment in this study suggest that there is no acute myocardial cell death after irradiation. Similarly, a normal value of NT-proBNP in all patients 12-months after surgery is correlated with absence of chronic cardiac damage induced by IORT.

Conclusion

In conclusion, IORT treatment, given intra-operatively with an IntraBeam device soon after local excision of the breast tumor, and delivering 20Gy of radiation directly onto the tumor bed, does not cause any acute or chronic heart damage detectable with TnI and NT-proBNP serum levels, respectively, bearing in mind that the heart of left-sided breast cancer patients was protected by a small sheet of tungsten. There were no differences in serum levels between patients treated for right or left breast cancer. These findings confirm the acute and chronic cardiac safety of IORT with IntraBeam. A larger patient population and a longer follow up will be necessary to finally confirm the cardiac tolerability of IORT.

Acknowledgement

The authors are grateful to the entire surgical, nursing and clinical staff.

References

1. Clarke M, Collins R, Darby S, *et al.* Breast Cancer Trialists' Collaborative Group (EBCTCG). Effects of radiotherapy and of differences in the extent of surgery for early breast cancer on local recurrence and 15-year survival: an overview of the randomised trials. Lancet 2005; 366: 2087-106.
2. Vaidya JS, Baum M, Tobias JS, *et al.* Long-term results of targeted intraoperative radiotherapy (Targit) boost during breast-conserving surgery. Int J Radiat Oncol Biol Phys 2011; 81:1091-7.
3. Vaidya JS, Joseph DJ, Tobias JS, *et al.* Targeted intraoperative radiotherapy versus whole breast radiotherapy for breast cancer (TARGIT-A trial): an international, prospective, randomised, non-inferiority phase 3 trial. Lancet 2010; 376: 91-102.
4. Vaidya JS, Wenz F, Bulsara M, *et al.* Risk-adapted targeted intraoperative radiotherapy versus whole-breast radiotherapy for breast cancer: five-year results for local control and overall survival from the TARGIT-A randomized trial. Lancet 2014; 383: 603-13.
5. Thygesen K, Mair J, Katus H, *et al.* Study Group on Biomarkers in Cardiology of the ESC Working Group on Acute Cardiac Care. Recommendations for the use of cardiac troponin measurement in acute cardiac care. Eur Heart J 2010; 31: 2197-204.
6. Nellessen U, Zingel M, Hecker H, *et al.* Effects of radiation therapy on myocardial cell integrity and pump function: which role for cardiac biomarkers? Chemotherapy 2010; 56: 147-52.
7. Hughes-Davies L, Sacks D, Rescigno J, *et al.* Serum cardiac troponin T levels during treatment of early-stage breast cancer. J Clin Oncol 1995; 13: 2582-4.
8. D'Errico MP, Grimaldi L, Petruzzelli MF, *et al.* N-terminal pro-B-type natriuretic peptide plasma levels as a potential biomarker for cardiac damage after radiotherapy in patients

with left-sided breast cancer. Int J Radiat Oncol Biol Phys 2012; 82: e239-46.
9. Erven K, Florian A, Slagmolen P, *et al.* Subclinical Cardiotoxicity Detected by Strain Rate Imaging up to 14 months After Breast Radiation Therapy. Int J Radiat Oncol Biol Phys 2013; 85: 1172-8.
10. Brosius FC 3rd, Waller BF, Roberts WC. Radiation heart disease. Analysis of 16 young (aged 15 to 33 years) necropsy patients who received over 3,500 rads to the heart. Am J Med 1981; 70: 519-30.
11. Boivin JF, Hutchison GB, Lubin JH, *et al.* Coronary artery disease mortality in patients treated for Hodgkin's disease. Cancer 1992; 69: 1241-7.
12. Hancock SL, Tucker MA, Hoppe RT. Factors affecting late mortality from heart disease after treatment of Hodgkin's disease. JAMA 1993; 270: 1949-55.
13. Heidenreich PA, Schnittger I, Strauss HW, *et al.* Screening for coronary artery disease after mediastinal irradiation for Hodgkin's disease. J Clin Oncol 2007; 25: 43-9.
14. Cuzick J, Stewart H, Rutqvist L, *et al.* Cause-specific mortality in long-term survivors of breast cancer who participated in trials of radiotherapy. J Clin Oncol 1994; 12: 447-53.
15. Shimizu Y, Pierce DA, Preston DL, *et al.* Studies of the mortality of atomic bomb survivors. Report 12, part II. Noncancer mortality: 1950-1990. Radiat Res 1999; 152: 374-89.
16. Rodemann HP, Bamberg M. Cellular basis of radiation-induced fibrosis. Radiother Oncol 1995; 35: 83-90.
17. Marks LB, Yu X, Prosnitz RG, *et al.* The incidence and functional consequences of RT-associated cardiac perfusion defects. Int J Radiat Oncol Biol Phys 2005; 63: 214-23.
18. Kozak KR, Hong TS, Sluss PM, *et al.* Cardiac blood biomarkers in patients receiving thoracic (chemo)radiation. Lung Cancer 2008; 62: 351-5.
19. Eisenman A. Troponin assays for the diagnosis of myocardial infarction and acute coronary syndrome: where do we stand? Expert Rev Cardiovasc Ther 2006; 4: 509-14.
20. Babuin L, Jaffe AS. Troponin: the biomarker of choice for the detection of cardiac injury. CMAJ 2005; 173: 1191-202.

Received: 19.05.2014
Accepted: 28.07.2014
Address: Tania Saibene, MD
Veneto Institute of Oncology
IOV - IRCCS, Padua, Italy
Via Gattamelata 64, 35128 Padova, Italy
Tel. +39 0498215550
Fax +39 0498215542
E-mail: tania.saibene@ioveneto.it